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Carole Heilman (Division of AIDS at the National Institutes of Allergy and Infectious Diseases) and David Baltimore (President of the California Institute of Technology) review current research efforts toward developing anti-HIV vaccines. While recognizing the extraordinary challenges involved in developing a vaccine, Heilman and Baltimore stress that important steps have been taken and that as such there is room for optimism.

HIV vaccines—where are we going?

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Despite the often voiced frustration that HIV vaccine research and development is static, a closer look at this area of research suggests that not only is recent technology and research impacting vaccine products currently under clinical development, but novel approaches are beginning to emerge. This review charts the impact of recent research findings that are influencing HIV vaccine design.

The humoral response

HIV vaccine products fall into two general categories, those that stimulate the humoral immune system and those that elicit a cellular immune response. With a focus on maximizing neutralizing antibody responses, the surface protein of HIV (Env) has been the main target of an antibody-based vaccine approach for two important reasons: First, it is the principal viral determinant that interacts with the host receptors; second, it is the major antigenic determinant to which neutralizing antibodies are directed. Although the tremendous variability among HIV isolates was a known problem with regard to developing an antibody based vaccine strategy, the main concern was the lack of cross-reactivity among the different HIV sequence based subsets (clades). The potential impact of other variations, such as HIV Env conformation, had not been fully appreciated until relatively recently. Opportunities to study the impact of Env conformation on HIV variation emerged shortly after the identification of a second host receptor, required for the binding and entry of HIV into host cells. CXCR4 and CCR5, two members of the chemokine receptor family, were identified as the prototypic second receptors.

HIV primary isolates are capable of infecting T cells and macrophages. Whereas, T cell line-adapted laboratory isolates (TCLA) use the CXCR4 receptor, isolates capable of infecting macrophages (M-tropic) use CCR5 (ref. 1). (The new nomenclature for these viruses are based on co-receptor usage. R5 viruses use CCR5 but not CXCR4; X4 viruses use CXCR4 but not CCR5; and R5X4 viruses can use both co-receptors.) The possible relevance of this finding to HIV vaccine design became apparent when considered in the context of two points: The vast majority of HIV isolated from patients with primary infection, infected macrophages (R5) (ref. 2); and X4, rather than R5, Env had been used to develop the various Env based vaccines. The use of X4 HIV isolates as Env vaccine candidates had made sense because they could readily generate high levels of neutralizing antibodies[3]. On closer examination however, the antibodies generated were restricted in their neutralizing activity to a small subset of viral isolates closely related to the vaccine virus[4]. Of even more concern was the lack of neutralizing cross reactivity with viruses isolated from patients. Now, by probing the HIV surface structure with monoclonal antibodies (mAbs), the inaccessibility of crucial epitopes within the Env of R5 viruses compared to X4 viruses is becoming clearer.

The mature Env protein exists as a processed, mature trimer on the surface of HIV. This protein is initially made as individual glycoproteins (gp160), which are cleaved into gpl20 and gp4l entities that non-covalently associate into the surface glycoprotein spikes[5]. Recent reports[6] further clarify the importance of the variable regions of gp120 in shielding the conserved binding regions from the immune system. Also shielding immunogenic epitopes from view is a complex of carbohydrates. In SIV (simian immunodeficiency virus)- infected rhesus monkeys the presence of these surface carbohydrates has been observed to directly impact the production of neutralizing antibodies. Infection with mutant forms of SIV lacking specific N-linked glycosylation sites resulted in markedly increased levels of neutralizing antibodies compared with the carbohydrate-sheltered native SIV[7].

The protective value of neutralizing antibodies generated by X4 Env-based vaccines has been hotly debated. The lack of relationship between neutralizing antibodies generated by X4 viruses and either containment of viral load in HIV-infected persons[8,9] or association with long-term non-progressors (LTNPs)[10] has been noted. On the other hand, neutralizing antibodies against R5 viruses, although difficult to detect in LTNPs, are often[11–13] but not always[14] found in these cohorts and are usually associated with subsets of LTNPs who have lower viral load[11].

Three human monoclonal antibodies (b12, 2G12 and 2F5) that broadly neutralize a panel of T- and M-tropic viruses have, however, been immortalized from the sera of infected patients. Each of these monoclonals can neutralize a genetically diverse range of R5 and X4 viruses from different clades (A–F) [15–17]. The importance of antibodies like these in preventing HIV infection or modulating disease course is still unclear. In passive transfer experiments, b12 administered at high doses to hu-PBL-SCID mice (severe combined immunodeficient mice populated with human peripheral blood mononuclear cells), completely protected these animals against R5 virus challenge.[18] However, such antibody had no impact at lower doses and could not affect the course of an ongoing infection. The rarity of antibodies that exhibit a cross clade response, has led some investigators to try to structurally define epitopic sites to which these antibodies are directed, with the hopes of developing a novel immunogen.

The ability to elicit high affinity, broadly reactive (that is, against multiple genetic variants) antibodies that can contain the virus, is a central theme in antibody-based vaccine design and development studies. For example, R5, gp120-based vaccines are being evaluated for their ability to elicit cross reacting neutralizing antibodies in Phase I studies. Unfortunately, a preliminary report[19] suggests that vaccination with an R5X4 gp120 vaccine did not broaden the neutralization response in vaccinees. The impact of conformation on directing the generation of protective antibodies is being addressed through development of gp140 (soluble oligomer containing gpl20 and the ectodomain of gp4l) and gpl60 vaccine candidates (complete gp120 and gp41sequence). The impact of adjuvants in the presentation of oligomeric candidates is also being evaluated. Using the SHIV/macaque model system (SHIV is a chimera virus containing the internal genes of SIV and the external HIV proteins), oligomeric gp160 formulated with either Ras3C®

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or polyphosphazene resulted in antibodies that neutralize a range of HIV isolates. Animals vaccinated with this immunogen and challenged with the homologous chimera (SHIV MN), were virus-free at four weeks post-challenge, whereas all non-vaccinated control animals were virus positive[20]. The importance of surface structure and presentation is also being considered through the development of pseudovirions, VLPs (viral-like particles), whole killed and other novel antigen display vehicles (for example, poliovirus, VEE and VSV vectors, as well as replicons—see Liu, page 515). Finally, consideration is being given to replicating a stable conformation, allowing for maximal expression of neutralizable epitopes based on the tertiary structure of the gp120-CD4-CCR5 interface.

The cellular response

Targeting the other arm of the immune system, cellular immunity, has always been and continues to be a major focus of HIV vaccine development. Various parts of the HIV genome have been expressed using live viral vectors such as vaccinia and avipox. With these vaccines, both non-human primates and humans have developed cytotoxic T lymphocytes (CTL) against the HIV encoded proteins and a recently developed method, the tetramer assay, is providing the technology to easily characterize the phenotypic specificity of the T cell response[21].

In humans, one of the most studied vectors has been a canarypox vector containing HIV $Env_{120\ \&\ TM}$+Gag+Pro+Pol sequences (vCP205). A CTL response, which usually ranges from approximately 20–45% at any single time point during a study, with 40–60% of volunteers mounting some response, can be demonstrated. Additionally, the duration of the CTL response in the majority of CTL-positive volunteers is at least 15 months after the last vaccination. The generation of CTLs by vCP125 containing Env gp160 vaccinated volunteers is of particular interest in that some were also capable of lysing autologous $CD4^+$ cells infected with HIV clade A–F isolates, suggesting a broad CTL response was induced[22].

In order to predict potential CTL cross-reactivity, amino acid sequences from HIV isolates of different clades have been compared with the sequence of functionally recognized clade B CTL epitopes. Over 65% of such clade B CTL epitopes are either identical or have a one amino acid difference with respect to the corresponding sequences in over 40 viruses from clades A, C, D, F, G or H, or interclade recombinants. Evaluation of CTLs from clade B infected individuals has shown cross-recognition among other clades[23–25]. In general, one amino acid difference from the primary peptide did not preclude recognition and often even two differences were tolerated. As expected, most of the differences across clades lie in gp160 (only ~ 45% of the gp160 CTL epitopes are identical or have one amino acid difference). Similar observations have been made when comparing T-cell help epitopes identified from clade B infections with corresponding sequences of other viruses. This analysis supports the concept that a 'monovalent' HIV vaccine might induce CTL activity (and T-cell help) against non-clade B viruses, a concept beginning to emerge from vaccine studies in Caucasian volunteers[22].

The critical question is whether CTL epitopes in a vaccine representing a given clade would be recognized by individuals of a different genetic background who were exposed to viruses of different clades. Such a concept will soon be tested in Uganda, where the vCP205 will be tested for its ability to induce CTL activity against other HIV clades.

More recently, two new vectors containing either $Env_{120\ \&\ TM}$+Gag+Pro+Pol and Nef CTL epitopes (vCP 1433) or vCP 1452—which contains $Env_{120\ \&\ TM}$+Gag+Pro+Pol and Nef CTL epitopes plus two vaccinia coding sequences (E31 and K31, which enhance, *in vitro*, the efficiency of HIV mRNA translation)—are planned for evaluation in Phase I trials, with expansion of the breadth and duration of the HIV specific CTL response, the obvious goal. Alternative HIV vaccine approaches for inducing CTL responses continue to be explored. Although lone peptides do not elicit a strong CTL response, approaches focused on presenting an array of HIV peptide, as branched-chain multimers or in combination with various adjuvants, for example, are being pursued for potential CTL boost strategies. Still under refinement for maximal expression and presentation, HIV DNA vaccines have shown tremendous promise as a way to induce strong CTL responses in animal model systems[26,27].

The combined approach

A third approach, receiving much attention, is to combine both humoral and cellular responses. The 'prime-boost strategy', which includes the administration of live recombinant and envelope based vaccines, in combination or in sequence, does not interfere with the individual responses seen when either vaccine is given alone. Indeed, the effects from the two vaccines appear to be additive when other components of immune responsiveness are measured. For example, vCP205 in combination with SF2gp 120 (from R4 virus) resulted in increased antibody dependent cellular cytotoxicity (ADCC) and T-cell help activities when compared to vCP205 given alone. Evidence for the possible role of ADCC and T-cell help in protection against HIV disease is only now emerging. HIV specific antibodies that mediate ADCC are found very early in acute infection and correlate well with declines in plasma virus[28,29]. Now, the importance of maintaining $CD4^+$ T-cell help during the containment of viral replication is emerging as a strong *in vivo* correlate[30]. Recent evaluation of a well-defined cohort with wide ranges of HIV viral loads and CD4 lymphoproliferative responses demonstrated that the ability to maintain strong CTL responses and control plasma viremia was directly correlated to the presence of p24 specific $CD4^+$ helper cells[31].

Another approach that appears to broadly target the immune system and has also received much attention, is the live, attenuated HIV vaccine. Studies of an SIV model with nef deletions have shown impressive protection[32]. Using a systematic approach for deleting regions of SIV, a series of potential SIV vaccines have been generated and evaluated in rhesus macaques. Evaluation of a highly attenuated version devoid of nef, vpr and upstream sequences in U3, SIV delta 3, demonstrated the presence of measurable CTLs, and increasing levels of SIV-neutralizing antibodies. More importantly, the animals were protected against intravenous challenge[32]. Evaluation of an HIV-infected Australian Blood Bank cohort described six individuals with a common source of infection, all of whom had remained disease-free for 10–14 years[33]. The virus isolated from each of these people showed a deletion in nef and a portion of the LTR, suggesting a naturally occurring attenuation.

Although the immune basis of the protective response of this approach is still not clear, the effectiveness of the approach has been proven in other infectious disease models. The Sabin polio vaccine, vaccinia, rubella, mumps and measles vaccines and more recently varicella, rotavirus and cold-adapted influenza vaccines all work by mimicking the natural infection process without inducing concomitant disease. Based on using a weakened strain of virus obtained by host restriction, selective mutations or deletions, or natural adaptation under restrictive growth conditions, these vaccines have all been found to be effective (see Hilleman, page 507). Lingering concerns about potential reversion to pathogenic strains

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has made the question of safety paramount and is probably behind the lack of aggressive development of attenuated HIV for use in humans. Dose-related pathogenicity was reported in neonatal monkeys vaccinated with SIV delta 3 nef[34,35]. More recently, long-term safety concerns have been raised as early clinical symptoms of AIDS have begun to be identified in monkeys vaccinated with attenuated SIV strains more than two years ago. Alternative live attenuated non-human lentivirus approaches are also being evaluated. Shibata and colleagues recently described the cross-protective response of attenuated SIV by challenging rhesus macaques with SHIV. Despite the absence of neutralizing antibodies, animals were protected against the chimera expressing human Env + Gag, suggesting the possibility that a non-human primate lentivirus may be a candidate vaccine for humans[36].

Better models

Parallel to the development of new HIV vaccines, opportunities to more rapidly evaluate their potential value are being explored. To date, the most used model for evaluating candidate vaccines is the SIV/macaque model. Although only 3–4 macaques per group have been used in most experiments, this model has provided valuable information regarding the potential success of various vaccine strategies. In general, protection against challenge by SIV strains that are poorly pathogenic has been achieved with a number of vaccines. However, protection against highly pathogenic SIV strains has only been seen in animals receiving live, attenuated SIV vaccines. This does not mean that other vaccine designs are doomed to fail but rather points to the paucity of SIV vaccines available for detailed evaluation and the evolving knowledge on the use of the SIV/macaque model. The recent generation of SHIV chimeras (that allow HIV-based envelope vaccines to be evaluated in an SIV model) and the development of corresponding pathogenic challenge viruses, is now providing an opportunity to evaluate in more detail a variety of HIV-based vaccines in macaques.

The recent development of smaller and less expensive animal models for the testing of HIV vaccines is interesting. Most intriguing are transgenic mice and rabbits expressing human CD4 and the relevant chemokine receptors. The rabbit model is unique in that it supports the replication of HIV, albeit at low levels[37,38], and preliminary reports (Speck and colleagues) suggest that replication levels can be increased through the expression of appropriate chemokine receptors.

There is a reason for optimism. The science discussed here is both exhilarating and sobering and the complexities of host/virus interactions present remarkable challenges. However, these are being addressed. We believe that expanded commitments from both government and the scientific community will hasten that pace.

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